

Call Characteristics of Two Sympatric and Morphologically Similar Tree Frogs Species, *Polypedates megacephalus* and *Polypedates mutus* (Anura: Rhacophoridae), from Hainan, China

Qiucheng LIU, Tongliang WANG, Xiaofei ZHAI and Jichao WANG*

Ministry of Education Key Laboratory for Tropical Island Ecology, College of Life Sciences, Hainan Normal University, Haikou 571158, China

Abstract Anuran calls are usually species-specific and therefore valued as a tool for species identification. Call characteristics are a potential honest signal in sexual selection because they often reflect male body size. *Polypedates megacephalus* and *P. mutus* are two sympatric and morphologically similar tree frogs, but it remains unknown whether their calls are associated with body size. In this study, we compared call characteristics of these two species and investigated any potential relationships with body size. We found that *P. megacephalus*, males produced six call types which consisting of three distinct notes, while *P. mutus* males produced five types consisting of two types of notes. Dominant frequency, note duration, pulse duration, and call duration exhibited significant interspecific differences. In *P. megacephalus*, one note exhibited a dominant frequency that was negatively correlated with body mass, snout-vent length, head length, and head width. In *P. mutus*, the duration of one note type was positively correlated with body mass and head width. These differences in call characteristics may play an important role in interspecific recognition. Additionally, because interspecific acoustic variation reflects body size, calls may be relevant for sexual selection. Taken together, our results confirmed that calls are a valid tool for distinguishing between the two tree-frog species in the field.

Keywords body size, call characteristics, *Polypedates*, sexual selection

1. Introduction

Amphibian communication is primarily dependent on vocalizations (Gerhardt and Huber, 2002; Cui *et al.*, 2011). In most anurans, acoustic interactions are key to male-male competition and female choice (Narins *et al.*, 2006; Xu *et al.*, 2011). The spectral and temporal parameters of acoustic calls are species-specific, and hence can be used for species identification and for determining phylogenetic relationships (Jiang *et al.*, 2002; Heyer and Barrio-Amorós, 2009). Thus, acoustic studies are an important tool for clarifying the taxonomy of sympatric and morphologically similar species.

Body size may affect the outcomes of sexual selection through male-male competition and female choice. Larger males usually defeat smaller males in aggressive

encounters, and females often preferentially choose larger males (Wells, 1978; Given, 1988; Richardson *et al.*, 2010; Liao and Lu, 2011; Rausch *et al.*, 2014). Previous studies have shown that the spectral and temporal parameters of calls could reflect frog body size (Martin, 1972; Gerhardt, 1994; Wang *et al.*, 2012; Wei *et al.*, 2012; Zhu *et al.*, 2016a). However, other studies suggest that acoustic properties and morphological characteristics are unrelated in some frog species (Penna, 2004; Márquez *et al.*, 2005).

Polypedates megacephalus and *P. mutus* (family Rhacophoridae) are tree frogs common throughout southern China and Southeast Asia (Fei *et al.*, 2012). The two species have controversial taxonomic status. In China, Hong Kong populations previously considered to be *P. leucomystax* were named as *P. megacephalus*, a new species (Hallowell, 1861), a classification that subsequent taxonomists conferred to all *P. leucomystax* populations in continental China (Matsui *et al.*, 1986; Zhao and Adler, 1993; Fei, 1999; Fei *et al.*, 2005, 2009). *Polypedates mutus* was identified from Hekou Yunnan,

* Corresponding author: Dr. Jichao WANG, from Hainan Normal University, Haikou, China, with his research focusing on behavioral ecology of amphibians and reptiles.

E-mail: wjc@hainnu.edu.cn

Received: 4 April 2018 Accepted: 27 November 2018

China, based on samples with a “striped” morphology and the lack of a vocal sac (Liu *et al.*, 1960); this species was later found throughout southern China (Inger *et al.*, 1999). Recent phylogenetic and taxonomic evidence supports the presence of *P. megacephalus* and *P. mutus* in southern China, including Hainan (Kuraishi *et al.*, 2012; Pan *et al.*, 2013). Acoustic call characteristics of both species have been previously described (Xu, 2005), but limited samples allowed only a few simple analyses of acoustic parameters and structure. A later study identified three note types in the complex call of *P. megacephalus* from Hong Kong (type locality) (Kuraishi *et al.*, 2011). However, no comprehensive comparison of call characteristics has been made between the two species thus far. It also remains unknown whether their calls reflect body size. This missing information complicates field identification of these two sympatric and morphologically similar species.

The objective of the present study was to compare the spectral and temporal parameters of vocalizations from sympatric *P. megacephalus* and *P. mutus*. We investigated

the relationship between call characteristics and male body size. Our study contributes to existing knowledge of vocal repertoires across tree frog species and provides comprehensive data that will help to distinguish interspecific call types.

2. Materials and Methods

2.1. Study species Snout-vent lengths of adult *P. megacephalus* males and females are 41–48 and 57–65 mm, respectively (Table 1). No black or brown spots are present on the sides of the body, but spots are arranged in reticulate patterns along the posterior (Figure 1A). Numerous small reticulate structures are present on the back (Figure 1C) (Liu *et al.*, 2018). Hind legs are stout and tibial-tarsal joints stretch between the eyes and nostrils. Male frogs have a pair of pharyngeal subcapsular structures. *Polypedates megacephalus* inhabits altitudes ranging between 80 and 2200 m (Fei *et al.*, 2012; Wang *et al.*, 2014).

Snout-vent lengths of adult males and females of *P.*



Figure 1 Morphological characteristics of *Polypedates megacephalus* (left) and *P. mutus* (right).

mutus are 52–63 and 53–77 mm, respectively (Table 1). Large and dark brown spots are arranged continuously on the sides of the body, and the posterior lacks any reticulate patterns (Figure 1B). A few large reticulate structures are present on the back (Figure 1D) (Liu *et al.*, 2018). Hind legs are slender, with tibial-tarsal joints stretching to the snout or nostrils, and male frogs lack a vocal sac. *Polypedates mutus* inhabits altitudes ranging between 340 and 1100 m (Fei *et al.*, 2012; Wang *et al.*, 2014).

2.2. Vocalization recording and analyses From June 10 to July 9, 2015, we recorded male *P. megacephalus* and *P. mutus* vocalizations in their original habitat of Mt. Diaoluo National Nature Reserve (18°43'22.02" N, 109°52'6.19" E, 933 m a.s.l.), Hainan, China. Recorded calls were always of isolated individuals and never from a mixed chorus. Vocalizations were recorded from 19:30 to 23:30 h (air temperature: 20.7 ± 1.4 °C, relative humidity: $94.5\% \pm 5.3\%$). Each recording lasted 10 min per male, using a directional microphone (Sennheiser ME-66 with K6 power module; Sennheiser, Wedemark, Germany) connected to a digital recorder (PMD-660, 16 bit, 44.1

kHz; Marantz, Kanagawa, Japan), placed approximately 1 m away from the subject. Calls of individuals from both species were recorded on the same night as far as possible. Actual species recorded was decided based on whichever individuals were found in one night. Data were saved as wav files.

Call parameters were defined and illustrated based on Köhler *et al.* (2017). Dominant frequency was sound energy concentrated within the whole power spectrum, while fundamental frequency was the base or lowest frequency band in each note. Acoustic parameters measured included the following temporal and spectral properties: note duration (ND), call duration (CD), note number (NN), pulse number (PN), pulse duration (PD), fundamental frequency (FF), and dominant frequency (DF). According to the recording quality, we selected 6–41 and 1–10 calls used for each individual of *P. megacephalus* and *P. mutus*, respectively. Oscillograms and spectrograms were prepared in PRAAT (version 5.1.11; Boersma and Weenink, 2017). Body mass, snout-to-vent length (SVL), head length (HL), and head width

Table 1 Body size and call properties of male *Polypedates megacephalus* and *P. mutus*.

<i>P. megacephalus</i>				<i>P. mutus</i>			
Parameter	Mean $\pm$ SD	Min–max	CV _b /CV _w	Parameter	Mean $\pm$ SD	Min–max	CV _b /CV _w
BM (g)	6.78 $\pm$ 1.25	4.88–8.91		BM (g)	12.20 $\pm$ 1.83	8.22–14.34	
SVL (mm)	53.38 $\pm$ 3.29	47.42–56.87		SVL (mm)	63.19 $\pm$ 3.89	55.40–68.86	
HL (mm)	17.79 $\pm$ 1.30	15.91–19.52		HL (mm)	20.35 $\pm$ 1.04	18.54–22.28	
HW (mm)	15.66 $\pm$ 1.31	13.10–17.84		HW (mm)	18.10 $\pm$ 1.10	16.11–19.82	
DFA (Hz)	1281.23 $\pm$ 315.23	559.80–2196.00	1.83	DFD (Hz)	1155.32 $\pm$ 123.10	602.90–1593.00	1.05
DFB (Hz)	1134.94 $\pm$ 353.94	645.90–2368.00	2.79	DFE (Hz)	1088.22 $\pm$ 111.69	645.90–1464.00	0.57
DFC (Hz)	1963.64 $\pm$ 110.35	1636.00–2239.00	1.24				
FFA (Hz)	227.86 $\pm$ 21.03	129.10–344.50	0.51	FFD (Hz)	209.44 $\pm$ 19.36	129.10–301.40	0.56
FFB (Hz)	213.81 $\pm$ 18.94	129.10–301.40	0.47	FFE (Hz)	217.65 $\pm$ 18.14	129.10–301.40	0.41
FFC (Hz)	236.65 $\pm$ 34.14	129.10–344.50	0.98				
NDA (ms)	26.08 $\pm$ 3.78	18.00–52.00	1.64	NDD (ms)	20.32 $\pm$ 1.93	13.00–28.00	1.09
NDB (ms)	124.16 $\pm$ 26.06	64.80–197.00	2.73	NDE (ms)	133.96 $\pm$ 29.95	89.00–120.00	2.29
NDC (ms)	30.15 $\pm$ 4.23	18.00–42.00	1.58				
NNA	6.01 $\pm$ 1.80	1.00–23.00	0.68	NND	14.14 $\pm$ 5.84	1.00–33.00	0.94
NNB	2.77 $\pm$ 1.14	1.00–9.00	0.71	NNE	3.00 $\pm$ 0.71	2.00–4.00	0.57
NNC	3.58 $\pm$ 0.58	2.00–4.00	1.76				
PNB	6.54 $\pm$ 1.07	3.00–11.00	1.11	PNE	6.98 $\pm$ 1.11	5.00–7.00	1.27
PDB (ms)	13.11 $\pm$ 1.00	4.00–24.00	0.41	PDE (ms)	16.60 $\pm$ 1.35	11.00–22.00	0.61
CD I (ms)	495.68 $\pm$ 145.72	21.00–1684.00	0.65	CD VII (ms)	3623.54 $\pm$ 1629.14	18.00–7731.00	0.97
CD II (ms)	723.02 $\pm$ 340.77	63.80–2670.60	0.55	CD VIII (ms)	698.33 $\pm$ 275.03	421.00–971.00	1
CD III (ms)	266.63 $\pm$ 65.15	111.00–416.00	1.76	CD IX (ms)	1864.67 $\pm$ 2066.80	566.00–4248.00	1.99
CD IV (ms)	911.43 $\pm$ 678.90	198.80–1890.50	2.46	CD X (ms)	4414.50 $\pm$ 1885.09	1385.00–9192.00	1.36
CD V (ms)	1714.77 $\pm$ 846.02	858.00–2812.00	1.06	CD XI (ms)	6484.58 $\pm$ 5076.90	2970.00–14094.00	0.71
CD VI (ms)	1631.30 $\pm$ 367.85	823.40–2764.00	0.93				

Note: A, B, and C are the three note types in *P. megacephalus*; D and E are the two note types in *P. mutus*. Abbreviations: BM: body mass; SVL: snout–vent length; HL: head length; HW: head width; DFA, DFB, DFC, DFD, DFE: dominant frequency of notes A, B, C, D, and E, respectively; FFA, FFB, FFC, FFD, FFE: fundamental frequency of notes A, B, C, D, and E, respectively; NDA, NDB, NDC, NDD, NDE: duration of (note A, B, C, D, and E); NNA, NNB, NNC, NND, NNE: respective number of notes A, B, C, D, and E renditions per call; PNB, PNE: pulse number of note B and E, respectively; PDB, PDE: pulse duration of note B and E, respectively; CD I–VI: call duration of call type I–VI in *P. megacephalus*; CD VII–XI: call duration of call type VII–XI in *P. mutus*.

(HW) were measured after the calls were recorded, respectively. Correlation analyses between morphological indexes and call parameters were conducted.

The ratio of CV_b (between-male coefficients of variation) to CV_w (within-male coefficients of variability) was calculated to find relative variability of between and within individuals' variation (Pettitt *et al.*, 2013; Fang *et al.*, 2018). If $CV_b/CV_w > 1.0$ for a call trait, there is more variability among males and this may have behavioral consequences for individual recognition (Bee *et al.*, 2001; Robisson *et al.*, 2010; Pettitt *et al.*, 2013). In the present study, when the number of calls > 1 from recorded individual, this subject was used to calculate CV_w .

2.3. Statistical analysis Data were statistically analyzed using SPSS 19.0 (IBM Corp., Chicago, IL, USA). To compare the differences of DF, FF or ND among the three note types in *P. megacephalus*, Friedman test was used. If the main effect was significant, Wilcoxon test was used to determine the difference for each parameter. Wilcoxon test was also conducted for each parameter in *P. mutus*. Mann-Whitney U tests were used to compare the differences in DF, FF, ND, CD, PN, and PD between species. Spearman's correlation analysis was used to detect possible relationships between vocalization characters and body size. Prior to statistical analyses, we calculated the average value for each call trait of each male frog, and then used those average values to calculate mean $\pm$ SD which represented the trait value of one species frog. Data are expressed as the mean $\pm$ SD, and $P < 0.05$ and $P < 0.001$ were considered statistically significant and highly statistically significant.

3. Results

3.1. Call characteristics Calls consisting of a single note type are simple calls, and calls consisting of different note types are complex calls (Köhler *et al.*, 2017). We recorded and analyzed spontaneous vocalizations from 18 *P. megacephalus* and 13 *P. mutus*. Male *P. megacephalus* produced six call types: I (note A), II (note B), III (note C), IV (note A + B), V (note B + A), and VI (note C + B), where notes A–C are three distinct note types. Male *P. mutus* produced five call types: VII (note D), VIII (note E), IX (note D + E), X (note E + D), and XI (note D + E + D), with notes D and E being distinct. Call waveforms and spectrograms are presented in Figures 2 and 3. The seven acoustic characteristics (DF, FF, ND, NN, PN, PD, and CD) are summarized in Table 1.

Polypedates megacephalus Call type I (Figure 2A) is a simple call, consisting of 1–23 (average 6.01 ± 1.80)

note-A renditions, CD ranging from 21.00–1684.00 ms ($n = 18$). The ND, DF, and FF of note A were 26.08 ± 3.78 ms ($n = 18$), 1281.23 ± 315.23 Hz ($n = 18$), and 227.86 ± 21.03 Hz ($n = 18$), respectively.

Call type II (Figure 2B) is a simple call, consisting of 1–9 (average 2.77 ± 1.14) note-B renditions, CD ranging from 63.80–2670.60 ms ($n = 18$). The ND, DF, and FF of note B were 124.16 ± 26.06 ms ($n = 18$), 1134.94 ± 353.94 Hz ($n = 18$), and 213.81 ± 18.94 Hz ($n = 18$), respectively. Calls exhibited 6.54 ± 1.07 pulses, with a PD of 13.11 ± 1.00 ms.

Call type III (Figure 2C) is a simple call, consisting of 2–4 (average 3.58 ± 0.58) note-C renditions, CD ranging from 111.00–416.00 ms ($n = 12$). The ND, DF, and FF of note C were 30.15 ± 4.23 ms ($n = 12$), 1963.64 ± 110.35 Hz ($n = 12$), and 236.65 ± 34.14 Hz ($n = 12$) respectively, with clear harmonics.

Call type IV (Figure 2D) is a complex call, with 2.00 ± 0.71 renditions of note A, followed by 2.00 ± 1.41 renditions of note B ($n = 4$).

Call type V (Figure 2E) is a complex call, with 2.75 ± 0.96 renditions of note B, followed by 8.75 ± 7.89 renditions of note A ($n = 4$).

Call type VI (Figure 2F) has 3.27 ± 0.70 renditions of note C, followed by 2.96 ± 1.20 renditions of note B ($n = 14$).

Type II calls were dominant across 18 individuals (57.43%), followed by type I (25.69%), type VI (7.50%), type III (6.60%), type IV (1.50%), and type V (1.30%). Friedman test results showed that DF and ND differed significantly among notes A, B, and C ($P < 0.001$), but FF ($P = 0.083$) did not. Wilcoxon tests revealed that DF differed significantly between notes A and C ($P < 0.002$), as well as note B and C ($P < 0.002$), but not between notes A and B ($P = 0.170$). Significant differences in ND were observed between notes A and B ($P < 0.001$), A and C ($P < 0.05$), as well as B and C ($P = 0.002$).

Polypedates mutus Call type VII (Figure 3A) is a simple call, consisting of 1–33 (average 14.14 ± 5.84) note-D renditions, CD ranging from 18.00–7731.00 ms ($n = 13$). The ND, DF, and FF of note D were 20.32 ± 1.93 ms ($n = 13$), 1155.32 ± 123.10 Hz ($n = 13$), and 209.44 ± 19.36 Hz ($n = 13$), respectively, with clear harmonics.

Call type VIII (Figure 3B) consisted of 2–4 (average 3.00 ± 0.71) note-E renditions, CD ranging from 421.00–971.00 ms ($n = 5$). The ND of note E was 133.96 ± 29.95 ms ($n = 5$), and its DF and FF were 1088.22 ± 111.69 Hz ($n = 5$) and 217.65 ± 18.14 Hz ($n = 5$), respectively, with 6.98 ± 1.11 pulses and PD was 16.60 ± 1.35 ms.

Call type IX (Figure 3C) is complex, with 7 ± 8.7

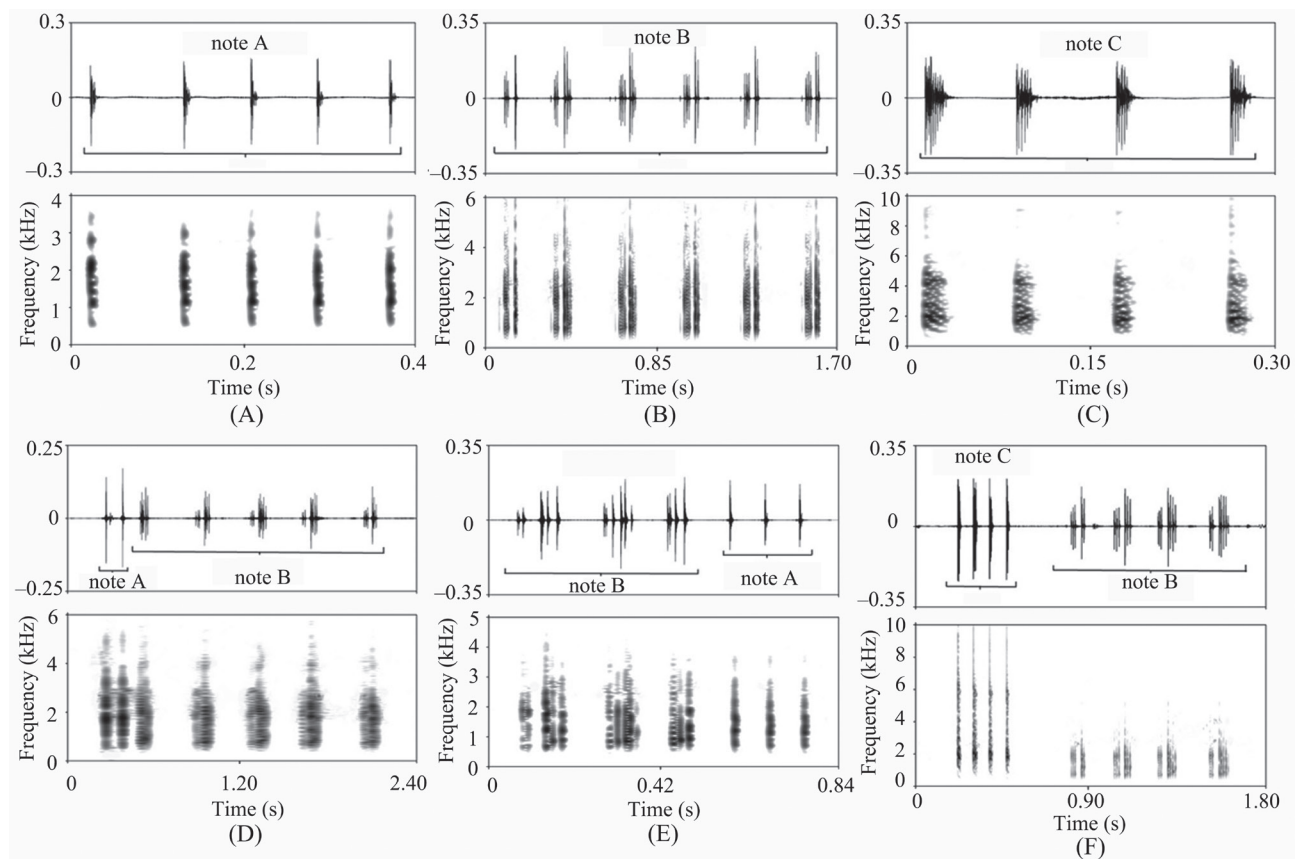


Figure 2 Amplitude-modulated waveforms and spectrograms of *Polypedates megacephalus* calls: (A) call type I, (B) call type II, (C) call type III, (D) call type IV, (E) call type V, and (F) call type VI.

renditions of note D, followed by 1.33 ± 0.58 renditions of note E ($n = 3$).

Call type X (Figure 3D) included 2.90 ± 0.74 renditions of note E, followed by 15.47 ± 8.98 renditions of note D ($n = 5$).

Call type XI (Figure 3E) was 12.33 ± 18.77 renditions of note D, followed by 1.67 ± 0.58 renditions of note E and 14.33 ± 3.51 renditions of note D ($n = 4$).

Call type VII was dominant across 13 individuals (66.67%), followed by type X (15.50%), type XI (9.50%), type IX (4.80%), and type VIII (3.60%). Wilcoxon tests revealed that note D and E differed significantly in ND ($P = 0.043$), but not in DF ($P = 0.345$) or FF ($P = 0.225$).

3.2. Comparisons of acoustic characteristics for simple calls in the two species of tree frogs The results of the Mann-Whitney U-test revealed no significant differences in DF, except between notes C and note D ($P < 0.001$), as well as notes C and note E ($P < 0.001$). Significant ND differences were observed between different notes of the two species, except between notes B and note E ($P = 0.371$). Excluding the comparisons between CD I and CD VIII ($P = 0.201$), CD I and CD IX ($P = 0.100$), CD II

and CD VIII ($P = 1.000$), CD II and CD IX ($P = 0.100$), CD III and CD VIII ($P = 0.109$), CD III and CD IX ($P = 0.109$), CD IV and CD VIII ($P = 1.000$), CD IV and CD IX ($P = 0.480$), CD V and CD VIII ($P = 0.157$), CD V and CD IX ($P = 1.000$), CD VI and CD VIII ($P = 0.105$), and CD VI and CD IX ($P = 0.487$), CD was significantly different between all other comparisons. Notes B and E did not differ significantly in PN ($P = 0.455$), but did in PD ($P = 0.001$). In *P. megacephalus* and *P. mutus*, $CV_b/CV_w > 1.0$ was 11 and 7 call traits, respectively (Table 1).

3.3. Relationship between body size and call structures

Correlation analysis was used to determine whether DF and ND were associated with body size. Mean male BM, SVL, HL, and HW values of *P. megacephalus* were 6.78 ± 1.25 g, 53.38 ± 3.29 mm, 17.79 ± 1.3 mm, and 15.66 ± 1.31 mm, respectively ($n = 11$) (Table 1). Only the DF of note B was negatively correlated with BM ($r = -0.665$, $P = 0.026$, $n = 11$; Figure 4A), SVL ($r = -0.763$, $P = 0.006$, $n = 11$; Figure 4B), HL ($r = -0.759$, $P = 0.007$, $n = 11$; Figure 4C), and HW ($r = -0.623$, $P = 0.040$, $n = 11$; Figure 4D). However, no significant correlation existed between ND and body size in *P. megacephalus* (Figure 4E–F).

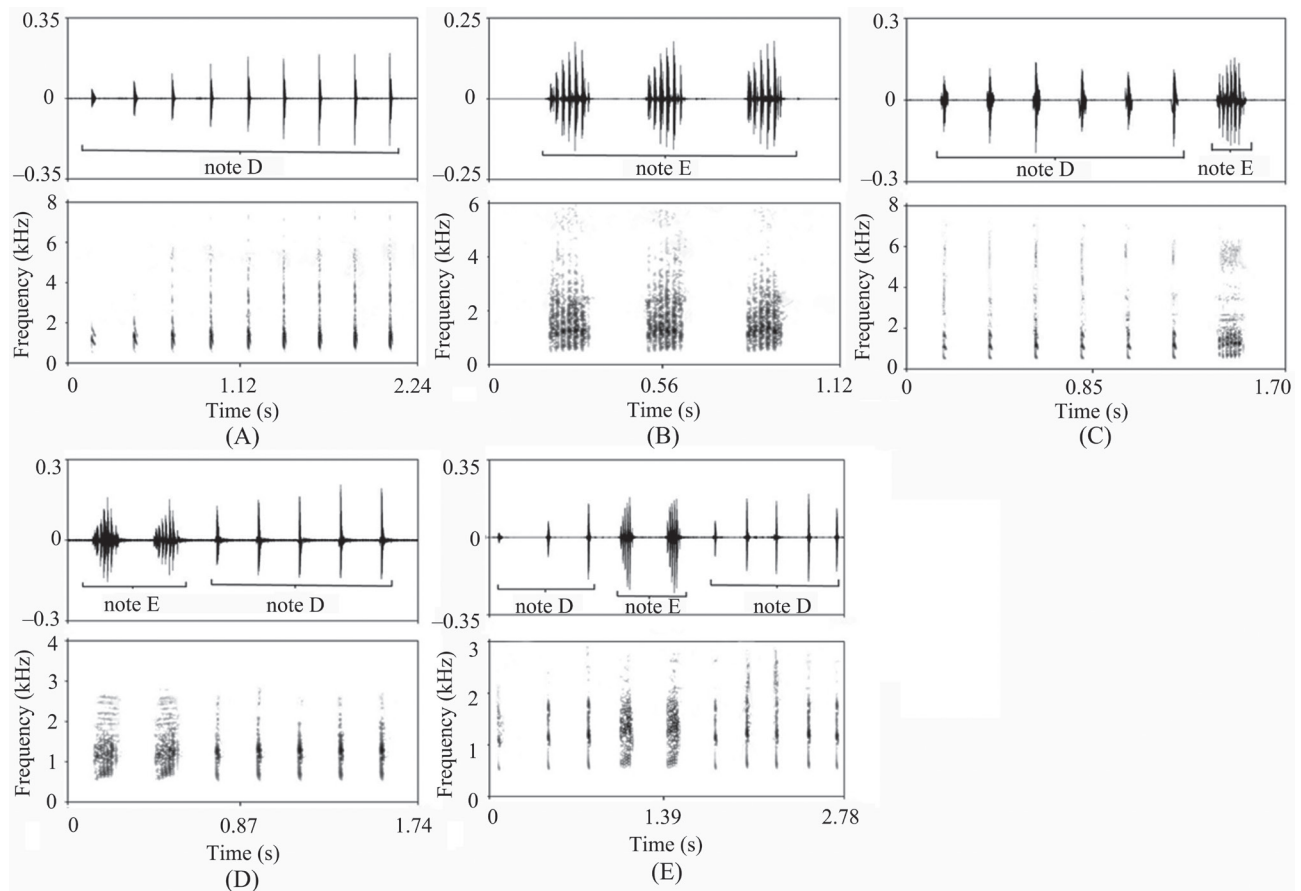


Figure 3 Amplitude-modulated waveforms and spectrograms of *Polypedates mutus* calls: (A) call type VII, (B) call type VIII, (C) call type IX, (D) call type X, and (E) call type XI.

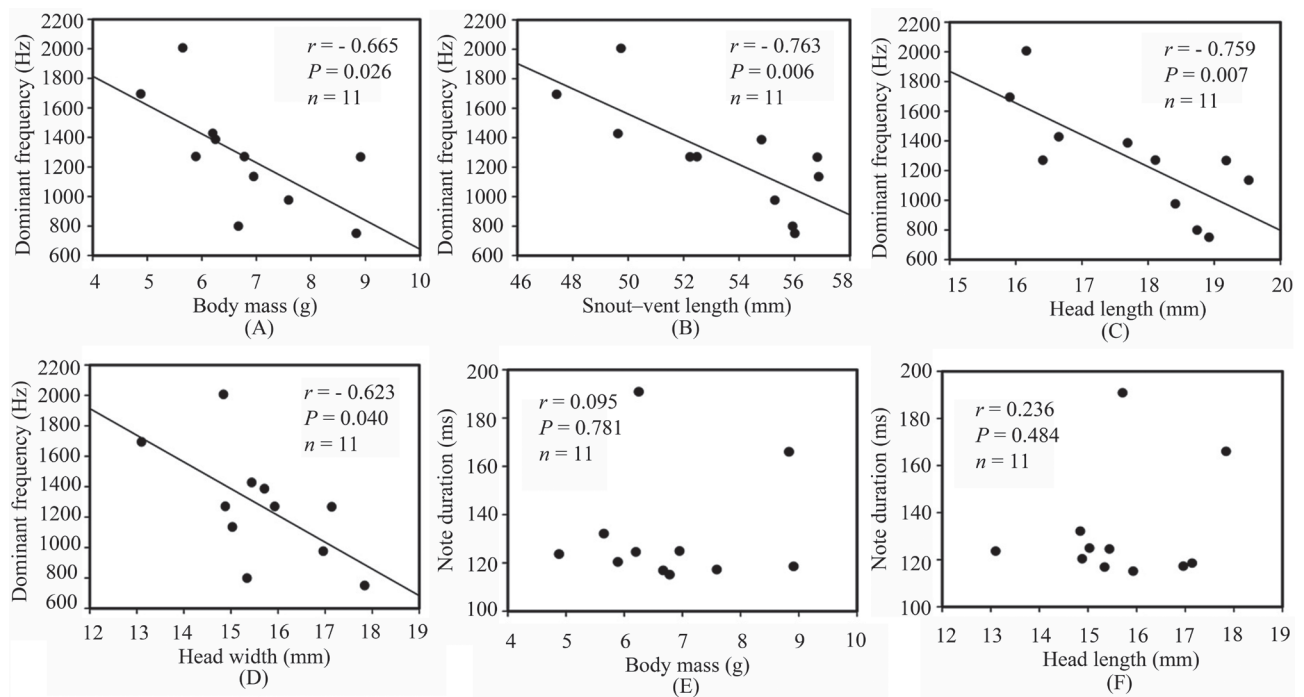


Figure 4 Relationship between dominant frequency of note B and body mass (A), snout-vent length (B), head length (C), and head width (D). Relationship between duration of note B and body mass (E) and head width (F) for *Polypedates megacephalus*.

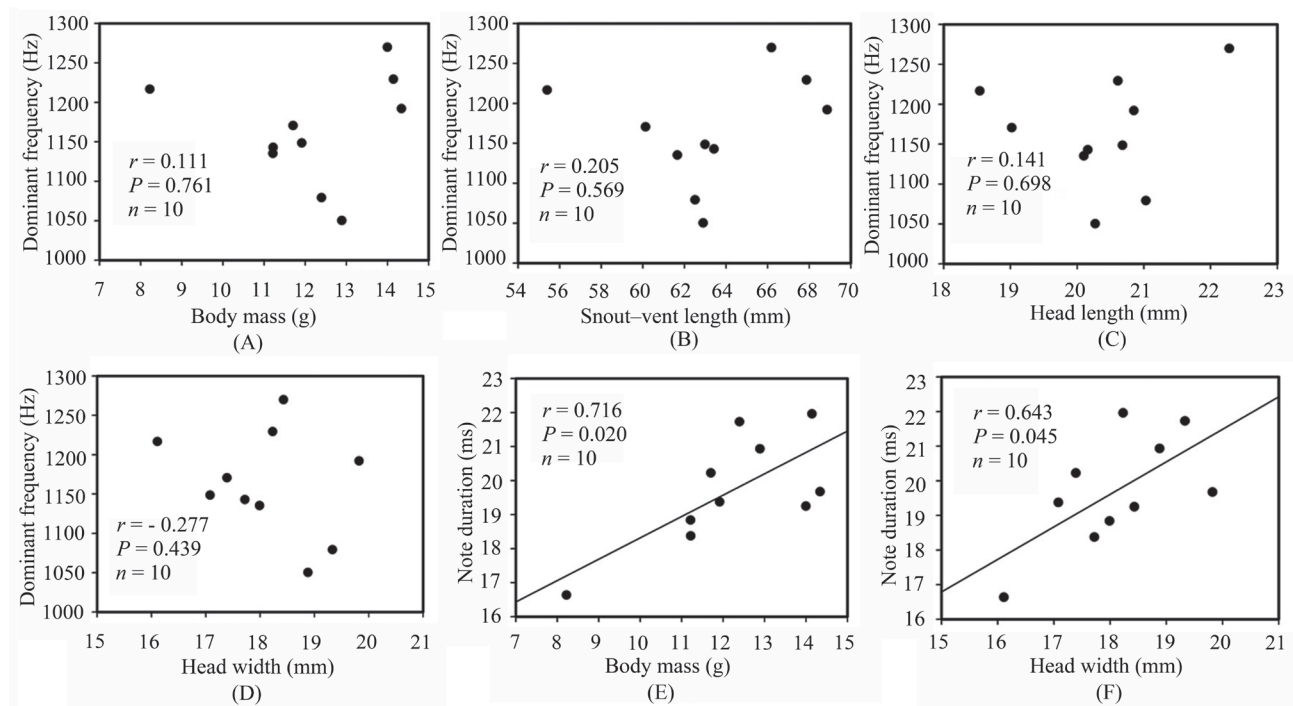


Figure 5 Relationship between dominant frequency of note D and body mass (A), snout-vent length (B), head length (C), and head width (D). Relationship between duration of note D and body mass (E) and head width (F) for *Polypedates mutus*.

Mean BM, SVL, HL, and HW of *P. mutus* males were 12.2 ± 1.83 g, 63.19 ± 3.89 mm, 20.35 ± 1.04 mm, and 18.1 ± 1.1 mm, respectively ($n = 10$) (Table 1). Only the ND of note D was positively correlated with BM ($r = 0.716$, $P = 0.020$, $n = 10$; Figure 5A) and HW ($r = 0.643$, $P = 0.045$, $n = 10$; Figure 5B), whereas DF was not significantly correlated with body size (Figure 5A–D).

4. Discussion

In the present study, calls from 18 *P. megacephalus* and 13 *P. mutus* were recorded. Calls of both species comprised multiple note types: three in *P. megacephalus* and two in *P. mutus*. In *P. megacephalus*, note C had harmonics, while notes A and B did not. In *P. mutus*, note D had harmonics, while note E did not. Because the two species differed in the proportion of each call type per unit time, and *P. mutus* was less abundant in the field, statistical between-species comparisons were only performed on simple call structure and their parameters. The investigation of between-species acoustic parameters revealed significant differences in DF, ND, PD, and CD. Similar results were also reported in two sympatric species of *Chiasmocleis*, suggesting that the observed differences could be due to variation in social behavior (Forlani *et al.*, 2013). In general, the difference in call characteristics reinforces existing species distinctions and

indicates that calls are important as a mechanism to avoid interspecific mating (Haddad *et al.*, 1994; Bastos *et al.*, 2011). Based on the described acoustic differences, the two species of tree frogs could be easily distinguished in the field.

Frogs and toads generally produce relatively simple calls consisting of a single note or a series of identical repeated notes (Wang *et al.*, 2016). Some anuran males, however, can produce dozens of complex calls. A previous study reported that the endemic *Boophis madagascariensis* possesses the largest known anuran vocal repertoire, with 28 distinct call types that differ in temporal pattern and spectral bandwidth (Narins *et al.*, 2000). *Polypedates leucomystax* in Southeast Asia produces nine different call notes and at least 12 different call types (Christensen-Dalsgaard *et al.*, 2002). In the present study, *P. megacephalus* produced more complex calls than did *P. mutus*. Previous research has suggested that in some anuran species, different note types have distinct functions (Kelley, 2004; Zhu *et al.*, 2017). Although we did not test the meaning of each call property via playback experiments, CV_b/CV_w values suggested that dominant frequency, note duration, note number, and call duration are important features useful for individual identification in both species.

Xu (2005) reported similar DF in a Guangxi population of *P. megacephalus*. However, calls from the two

populations differed in FF and ND. Previously reports of Hong Kong *P. megacephalus* call structure also had three note types, similar to our findings (Kuraishi *et al.*, 2011). The DF of Hainan *P. mutus* and a Guangxi population was similar, but FF differed between them (Xu, 2005). Thus, these findings suggest that geographic variation may exist in *P. megacephalus* and *P. mutus* calls. This hypothesis is being thoroughly tested in our ongoing research.

Call characteristics are correlated with body size in some species and are potentially important signals related to male-male competition and mate choice (Given, 1987; Morris and Yoon, 1989; Bee *et al.*, 1999; Gerhardt and Huber, 2002; Narins *et al.*, 2006; Zhu *et al.*, 2016b). In the present study, we found that the DF of note B was negatively correlated with body size in *P. megacephalus*, thus corroborating findings from many other anuran species and providing evidence that DF is a reliable indicator of male body size (Wang *et al.*, 2012, 2016; Zhu *et al.*, 2016a). However, note duration and body size were not significantly correlated in *P. megacephalus*, nor were dominant frequency and body size correlated in *P. mutus*. The ND of note D was positively correlated with BM and HW in *P. mutus*. This pattern probably occurs because longer ND results in longer call duration, which might be metabolically costly to produce and requires a larger body size (Wang *et al.*, 2012; Zhu *et al.*, 2016a). In conclusion, although the sample size was small for each species in the present study, the current results still suggest that interspecific acoustic differences could reflect body size and facilitate intraspecific identification, thereby playing an important role in sexual selection.

Limitations In the present study, calls in 18 individuals of *P. megacephalus* and 13 individuals of *P. mutus* were recorded to analyze the call characteristics. Because the proportion of each call type per unit time in both species was different, and the density of *P. mutus* was also lower in the field, only some call parameters were statistically compared between the two species of tree frogs.

Acknowledgements We thank Hao ZHANG for the help in field experiment. We are grateful to Zhixin SUN, Bicheng ZHU, and Longhui ZHAO for helpful and advice on analyses. This work was supported by the National Natural Science Foundation of China (31260518 to JW) and the Education Department of Hainan Province (00501023523).

References

- Bastos R. P., Signorelli L., Morais A. R., Costa T. B., Lima L. P., Jr J. P. P. 2011. Advertisement calls of three anuran species (Amphibia) from the Cerrado, Central Brazil. *S Am J Herpetol*, 6: 67–72
- Bee M. A., Kozich C. E., Blackwell K. J., Gerhardt H. C. 2010. Individual variation in advertisement calls of territorial male green frogs, *Rana clamitans*: implications for individual discrimination. *Ethology*, 107: 65–84
- Bee M. A., Perrill S. A., Owen P. C. 1999. Size assessment in simulated territorial encounters between male green frogs (*Rana clamitans*). *Behav Ecol Sociobiol*, 45: 177–184
- Boersma P., Weenink D. 2017. Praat: doing phonetics by computer [Computer program], Version 5.1.11. Available from: <http://www.praat.org/>
- Christensen-Dalsgaard J., Ludwig T. A., Narins P. M. 2002. Call diversity in an old world treefrog: level dependence and latency of acoustic responses. *Bioacoustics*, 13: 21–35
- Cui J. G., Tang Y. Z., Narins P. M. 2011. Real estate ads in Emei music frog vocalizations: Female preference for calls emanating from burrows. *Biol Lett*, 8: 337–340
- Fang K., Zhang B. W., Brauth S. E., Tang Y. Z., Fang G. Z. 2018. The first call note of the Anhui tree frog (*Rhacophorus zhoukaiya*) is acoustically suited for enabling individual recognition. *Bioacoustics*, DOI: 10.1080/09524622.2017.1422805
- Fei L. 1999. Atlas of Amphibians of China. China: Henan Scientific and Technologic Press, Zhengzhou (In Chinese)
- Fei L., Hu S. Q., Ye C. Y., Huang Y. Z. 2009. Fauna Sinica, Vol. 2, Amphibia. Science Press, Beijing China (In Chinese)
- Fei L., Ye C. Y., Jiang J. P., Xie F., Huang Y. Z. 2005. An Illustrated Key to Chinese Amphibians. Sichuan Science and Technology, Chengdu China (In Chinese)
- Fei L., Ye C. Y., Jiang J. P. 2012. Colored Atlas of Chinese Amphibians and Their Distributions. Sichuan Science and Technology Press, Chengdu (In Chinese)
- Forlani M. C., Mendes C. V. D. M., Dias I. R., Ruas D. S., Tonini J. F. R., Sá R. O. D. 2013. The advertisement calls and distribution of two sympatric species of *Chiasmocleis* (Méhely 1904) (Anura, Microhylidae, Gastrophryninae) from the Atlantic Forest. *S Am J Herpetol*, 8: 46–51
- Gerhardt H. C., Huber F. 2002. Acoustic communication in insects and anurans: Common problems and diverse solutions. University of Chicago Press, Chicago
- Gerhardt H. C. 1994. The evolution of vocalization in frogs and toads. *Annu Rev Ecol S*, 25: 293–324
- Given M. F. 1987. Vocalization and acoustic interactions of the carpenter frog, *Rana virgatipes*. *Herpetologica*, 43: 467–481
- Given M. F. 1988. Territoriality and aggressive interactions of male carpenter frogs, *Rana virgatipes*. *Copeia*, 1988: 411–421
- Hallowell E. 1861. Report upon the Reptilia of the North Pacific Exploring Expedition, under command of Capt. John Rogers, U.S. N. *P Acad Nat Sci Phila*, 12: 480–510
- Heyer W. R., Barrio-Amorós C. L. 2009. The advertisement calls of two sympatric frogs, *Leptodactylus lithonaetes* (Amphibia: Anura: Leptodactylidae) and *Pristimantis vilarsi* (Amphibia: Anura: Strabomantidae). *P Biol Soc Wash*, 122: 282–291
- Inger R. F. 1999. Distributions of amphibians in southern Asia and adjacent islands. Pp. 445–482. In Duellman W. E. (Ed.), *Patterns of distribution of amphibians: A global perspective*. Baltimore:

John Hopkins University Press

- Jiang J. P., Xie F., Fei L., Ye C. Y., Zheng M.** 2002. Mating calls of six forms of pleobatid in Wa Wu Mountain National Forest Park, Sichuan, China (Anura: Pelobatidae). *Zool Res*, 23: 89–94 (In Chinese)
- Kelley D. B.** 2004. Vocal communication in frogs. *Curr Opin Neurobiol*, 14: 751–757
- Kuraishi N., Matsui M., Hamidy A., Belabut D. M., Ahmad N., Panha S., Sudin A., Yong H. S., Jiang J. P., Ota H., Thong H. T., Nishikawa K.** 2012. Phylogenetic and taxonomic relationships of the *Polypedates leucomystax* complex (Amphibia). *Zool Scr*, 42: 54–70
- Kuraishi N., Matsui M., Ota H., Chen S. L.** 2011. Specific separation of *Polypedates braueri* (Vogt, 1911) from *P. megacephalus* (Hallowell, 1861) (Amphibia: Anura: Rhacophoridae). *Zootaxa*, 2744: 53–61
- Köhler J., Jansen M., Rodríguez A., Pjr K., Toledo L. F., Emmrich M., Glaw F., Haddad C. F. B., Rödel M. O., Vences M.** 2017. The use of bioacoustics in anuran taxonomy: Theory, terminology, methods and recommendations for best practice. *Zootaxa*, 4251: 1–124
- Liao W. B., Lu X.** 2011. Proximate mechanisms leading to large male mating advantage in the Andrew's toad, *Bufo andrewsi*. *Behaviour*, 148: 1087–1102
- Liu C. C., Hu S. Q., Yang F. H.** 1960. Amphibia of Yunnan collected in 1958. *Acta Zool Sin*, 12: 149–174
- Liu Q. C., Zhai X. F., Wang T. L., Wang J. C.** 2018. Morphological identification and diversity of *Polypedates* species (Rhacophoridae, Anura, Amphibia) on Hainan Island. *Sichuan J Zool*, 5(37): 490–496 (In Chinese)
- Márquez R., Penna M., Marques P., do Amaral J. P. S.** 2005. Diverse types of advertisement calls in the frogs *Eupsophus calcaratus* and *E. roseus* (Leptodactylidae): A quantitative comparison. *Herpetol J*, 15(4): 257–263
- Martin W. F.** 1972. Evolution of vocalization in the genus *Bufo*. Pp. 279–309. In Blair W. F. (Ed.), *Evolution in the Genus Bufo*. University of Texas Press, Austin
- Matsui M., Seto T., Utsunomiya T.** 1986. Acoustic and karyotypic evidence for specific separation of *Polypedates megacephalus* from *P. leucomystax*. *J Herpetol*, 20: 483–489
- Morris M. R., Yoon S. L.** 1989. A mechanism for female choice for large males in the treefrog *Hyla chrysoscelis*. *Behav Ecol Sociobiol*, 25: 65–71
- Narins P. M., Feng A. S., Fay R. R., Popper A. N.** 2006. *Hearing and sound communication in amphibians*. Springer Science, New York
- Narins P. M., Lewis E. R., McClell B. E.** 2000. Hyperextended call note repertoire of the endemic Madagascar treefrog *Boophis madagascariensis* (Rhacophoridae). *The Zoological Society of London*, 250: 283–298
- Pan S. L., Dang N. X., Wang J. S., Zheng Y. T., Rao D. Q., Li J. T.** 2013. Molecular phylogeny supports the validity of *Polypedates impresus* yang 2008. *Asian Herpetol Res*, 4: 124–133
- Penna M.** 2004. Amplification and spectral shifts of vocalizations inside burrows of the frog *Eupsophus calcaratus* (Leptodactylidae). *J Acoust Soc Am*, 116: 1254–1260
- Pettitt B. A., Bourne G. R., Bee M. A.** 2013. Advertisement call variation in the golden rocket frog (*Anomaloglossus beebei*): Evidence for individual distinctiveness. *Ethology*, 119: 244–256
- Haddad C. F. B., Pombal Jr. J. P., Batistic R. F.** 1994. Natural hybridization between diploid and tetraploid species of Leaf-frogs, Genus *Phyllomedusa* (Amphibia). *J Herpetol*, 28(4): 425–430
- Pröhl H.** 2003. Variation in male calling behaviour and relation to male mating success in the Strawberry poison frog (*Dendrobates piumilio*). *Ethology*, 109: 273–290
- Rand A. S., Ryan M.** 1981. The adaptive significance of a complex vocal repertoire in a neotropical frog. *Ethology*, 57: 209–214
- Rausch A. M., Sztatecsny M., Jehle R., Ringler E., Hödl W.** 2014. Male body size and parental relatedness but not nuptial colouration influence paternity success during scramble competition in *Rana arvalis*. *Behaviour*, 151: 1869–1884
- Pettitt B. A., Bourne G. R., Bee M. A.** 2013. Advertisement call variation in the Golden rocket frog (*Anomaloglossus beebei*): Evidence for individual distinctiveness. *Ethology*, 119: 244–256
- Richardson C., Joly P., Léna J. P., Plénet S., Lengagne T.** 2010. The challenge of finding a high quality male: A treefrog solution based on female assessment of male calls. *Behaviour*, 147: 1737–1752
- Robisson P., Aubin T., Bremond J. C.** 2010. Individuality in the voice of the emperor penguin *Aptenodytes forsteri*: Adaptation to a noisy environment. *Ethology*, 94: 279–290
- Smith M. J., Hunter D.** 2005. Temporal and geographic variation in the advertisement call of the Booroolong frog (*Litoria booroolongensis*: Anura: Hylidae). *Ethology*, 111: 1103–1115
- Wang J. C., Yang C. C., Lin L., Liu H. W.** 2014. *Wild Vertebrate In Diaoluoshan, Hainan, China*. China Forestry Press, Beijing (In Chinese)
- Wang J. C., Cui J. G., Shi H. T., Brauth S. E., Tang Y. Z.** 2012. Effects of body size and environmental factors on the acoustic structure and temporal rhythm of calls in *Rhacophorus dennysi*. *Asian Herpetol Res*, 3: 205–212
- Wang J. C., Wang T. L., Wang P. P., Cui J. G.** 2016. Calls characteristics in round-tongued floating *Phrynoglossus martensii*. *Chin J Zool*, 51: 214–220 (In Chinese)
- Wei L., Zhao L. H., Ma X. H., Fan X. L., Ma X. M., Lin Z. H.** 2012. Advertisement call variability in the black-spined toad *Bufo melanostictus* (Anura: Bufonidae) during the breeding season in Lishui, Zhejiang, China. *Asian Herpetol Res*, 3: 157–162
- Welch A. M., Semlitsch R. D., Gerhardt H. C.** 1998. Call duration as an indicator of genetic quality in male gray tree frogs. *Science*, 280: 1928–1930
- Wells K. D.** 1978. Territoriality in the green frog (*Rana clamitans*): Vocalizations and agonistic behaviour. *Anim Behav*, 26: 1051–1063
- Xiong L. C., Matsui M., Jiang J. P., Nishikawa K.** 2015. Advertisement calls of two horned frogs, *Megophrys kuatunensis* and *M. huangshanensis*, from China (Anura, Megophryidae). *Curr Herpetol*, 34: 51–59
- Xu F., Cui J. G., Song J., Brauth S. E., Tang Y. Z.** 2011. Male competition strategies change when information concerning female receptivity is available. *Behav Ecol*, 23: 307–312
- Xu J. X., Xie F., Jiang J. P., Mo Y. M., Zheng Z. H.** 2005. The acoustic features of the mating call of 12 anuran species. *Chin J*

Zool, 40: 12–19 (In Chinese)

Zhao E. M., Adler K. 1993. Herpetology of China. Oxford, Ohio: Society for the study of amphibians and reptiles in cooperation with Chinese. Society for the Study of Amphibians and Reptiles

Zhu B. C., Wang J. C., Cui J. G., Brauth S. E., Tang Y. Z. 2016a. The spectral structure of vocalizations match hearing sensitivity but imprecisely in *Philautus odontotarsus*. Bioacoustics, 121–134

Zhu B. C., Wang J. C., Zhao L. H., Sun Z. X., Cui J. G., Brauth S. E., Tang Y. Z. 2016b. Bigger is not always better: females prefer males of mean body size in *Philautus odontotarsus*. Plos One, 11(2): e0149879

Zhu B. C., Wang J. C., Zhao L. H., Chen Q., Sun Z. X., Yang Y., Brauth S. E., Tang Y. Z., Cui J. G. 2017. Male-male competition and female choice are differentially affected by male call acoustics in the serrate-legged small treefrog, *Kurixalus odontotarsus*. PeerJ, 5: e3980